université de BORDEAUX

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Python pour la biologie

Biopython

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BIOINFORMATIQUE

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What is Biopython ?

- The Biopython Project is an international association of developers of freely available Python (http://www.python.org) tools for computational molecular biology

- The Biopython web site (http://www.biopython.org) provides an online resource for modules, scripts, and web links for developers of Python-based software for bioinformatics use and research

- Basically, the goal of Biopython is to make it as easy as possible to use Python for bioinformatics by creating high-quality, reusable modules and classes

Biopython functionalities(1)

- The ability to parse bioinformatics files into Python utilizable data structures, including support for the following formats:
  - Blast output - both from standalone and WWW Blast
  - Clustalw
  - FASTA
  - GenBank
  - PubMed and Medline
  - ExPASy files, like Enzyme and Prosite
  - SCOP, including “dom” and “lin” files
  - UniGene
  - SwissProt

Biopython functionalities(2)

- Files in the supported formats can be iterated over record by record or indexed and accessed via a Dictionary interface.

- Code to deal with popular on-line bioinformatics destinations such as:
  - NCBI { Blast, Entrez and PubMed services
  - ExPASy { Swiss-Prot and Prosite entries, as well as Prosite searches

- Interfaces to common bioinformatics programs such as:
  - Standalone Blast from NCBI
  - Clustalw alignment program
  - EMBOSS command line tools

Les fonctionnalités Biopython (3)

- Standard sequence class that deals with sequences, ids on sequences, and sequence features.
- Performing common operations on sequences, such as translation, transcription and weight calculations.
- Perform classication of data using k-Nearest Neighbors, Naive Bayes or Support VectorMachines.
- Dealing with alignments, including a standard way to create and deal with substitution matrices.
- Making it easy to split up parallelizable tasks into separate processes.
- GUI-based programs to do basic sequence manipulations, translations, BLASTing, etc.
- Extensive documentation and help with using the modules, including this le, on-line wiki documentation, the web site, and the mailing list.

Working with sequence: The Seq Object

- Most of the time when we think about sequences we have in my mind a string of letters like `AGTACACTGGT'.
- You can create such Seq object with this sequence as follows the ">>>" represents the Python prompt followed by what you would type in
- DON'T FORGET TO USE => from Bio.Seq import Seq in your script

```
>>>my_seq = Seq("AGTACACTGGT")
>>>my_seq
```

```
Seq(AGTACACTGGT', Alphabet())
>>>  print(my_seq)
AGTACACTGGT
```

```
>>>my_seq.alphabet
Alphabet()
>>>my_seq.complement()
Seq(TCATGTGACCA', Alphabet())
>>>my_seq.reverse_complement()
Seq(ACCAGTGTACT', Alphabet())
```

Sequences et Alphabet: IUPAC Alphabet for DNA, RNA and proteins

- Available alphabets for Biopython are defined in the Bio.Alphabet module.
- IUPAC (http://www.chem.qmw.ac.uk/iupac/): Bio.Alphabet.IUPAC
  - Basic IUPACProtein class
  - Additional ExtendedIUPACProtein class with Additional elements:
    - "U" (or "Sec" for selenocysteine)
    - "O" (or "Pyl" for pyrrolysine)
  - Plus the ambiguous symbols:
    - "B" (or "Asx" for asparagine or aspartic acid)
    - "Z" (or "Glx" for glutamine or glutamic acid)
    - "J" (or "Xle" for leucine isoleucine)
    - "X" (or "Xxx" for an unknown amino acid).
  - IUPACUnambiguousDNA, which provides for just the basic letters
  - IUPACAmbiguousDNA, which provides for ambiguity letters for every possible situation
  - ExtendedIUPACDNA, which allows letters for modifiedbase

Sequences et Alphabet (2)

➔ Create an ambiguous sequence with the default generic alphabet:

```
>>> my_seq =  Seq("AGTACACTGGT”)
>>> my_seq
Seq('AGTACACTGGT', Alphabet())
>>> my_seq.alphabet
Alphabet()
```

➔ Specify the alphabet explicitly

```
>>> from Bio.Alphabet import IUPAC
>>> my_seq = Seq("AGTACACTGGT", IUPAC.unambiguous_dna)
>>> my_seq
Seq('AGTACACTGGT', IUPACUnambiguousDNA())
>>> my_seq.alphabet
IUPACUnambiguousDNA()
```

```
>>> from Bio.Alphabet import IUPAC
>>> my_prot = Seq("AGTACACTGGT", IUPAC.protein)
>>> my_prot
Seq('AGTACACTGGT', IUPACProtein())
>>> my_prot.alphabet
IUPACProtein()
```

Sequences act like strings (1)

- Deal with Seq objects as if they were normal Python strings
- For example getting the length, or iterating over the elements:

```
>>> from Bio.Alphabet import IUPAC
>>> my_seq = Seq("GATC", IUPAC.unambiguous_dna)
>>> for index, letter in enumerate(my_seq):
... print("%i %s" % (index, letter))
0 G
1 A
2 T
3 C

>>> print(len(my_seq))
4
```

- Access elements of the sequence in the same way as for string

```
>>> print(my_seq[0]) #first letter
G
>>> print(my_seq[2]) #third letter
T
>>> print(my_seq[-1]) #last letter
G
```

Sequences act like strings (2)

- TheSeq object has a ".count()" method, just like a string. Note that this means that like a Python string, this gives a non-overlapping count:

```
>>> "AAAA".count("AA")
2
>>> Seq("AAAA").count("AA")
2
```

- For some biological uses, you may actually want an overlapping count (i.e. 3 in this trivial example). When searching for single letters, this makes no difference:

```
>>> from Bio.Alphabet import IUPAC
>>> my_seq = Seq('GATCGATGGGCCTATATAGGATCGAAAATCGC', IUPAC.unambiguous_dna)
>>> len(my_seq)
32
>>>  my_seq.count("G")
9
>>>  100 * float(my_seq.count('G') + my_seq.count('C')) / len(my_seq)
46.875
```

Sequences act like strings (3)

- While you could use the above snippet of code to calculate a GC%, note that the Bio.SeqUtils module has several GC functions already built.

```
>>> from Bio.Seq import Seq
>>> from Bio.Alphabet import IUPAC
>>> from Bio.SeqUtils import GC
>>> my_seq = Seq('GATCGATGGGCCTATATAGGATCGAAAATCGC', IUPAC.unambiguous_dna)
>>> GC(my_seq)
46.875
```

- Note that using the Bio.SeqUtils.GC() function should automatically cope with mixed case sequences and the ambiguous nucleotide S which means G or C.
- Also note that just like a normal Python string, the Seq object is in some ways \read-only".
- If you need to edit your sequence, for example simulating a point mutation, you need to use a MutableSeq object).

Slicing a sequence

- Let's get a slice of the sequence

```
>>> from Bio.Seq import Seq
>>> from Bio.Alphabet import IUPAC
>>> my_seq = Seq("GATCGATGGGCCTATATAGGATCGAAAATCGC", IUPAC.unambiguous_dna)
>>> my_seq[4:12]
Seq('GATGGGCC', IUPACUnambiguousDNA())
```

- The new object produced is another Seq object which retains the alphabet information from the original Seq object
- Get the first, second and third codons positions using “stride” (“::”) :

```
>>> my_seq[0::3]
Seq('GCTGTAGTAAG', IUPACUnambiguousDNA())
>>> my_seq[1::3]
Seq(AGGCATGCATC',  IUPACUnambiguousDNA())
>>> my_seq[2::3]
Seq('TAGCTAAGAC', IUPACUnambiguousDNA())
>>> my_seq[::-1] ## Get the reverse sequence
Seq('CGCTAAAAGCTAGGATATATCCGGGTAGCTAG', IUPACUnambiguousDNA())
```

Turning Seq objects into strings

- To write to a file, or insert into a database

```
>>> str(my_seq)
'GATCGATGGGCCTATATAGGATCGAAAATCGC'
```

- Calling str() on a Seq object returns the full sequence as a string
- Python does this automatically in the print function

```
>>> print(my_seq)
GATCGATGGGCCTATATAGGATCGAAAATCGC
```

- Also use the Seq object directly with a %s placeholder when using the Python string formatting or interpolation operator (%)

```
>>>  fasta_format_string =“>Name\n%s\n“ % my_seq
>>> print(fasta_format_string)
>Name
GATCGATGGGCCTATATAGGATCGAAAATCGC
<BLANKLINE>
```

Concatenating or adding sequences

➔ Can't add sequences with incompatible alphabets, (protein and DNA)

```
>>> from Bio.Alphabet import IUPAC
>>> protein_seq = Seq("EVRNAK", IUPAC.protein)
>>> dna_seq = Seq("ACGT", IUPAC.unambiguous_dna)
>>> protein_seq + dna_seq

Traceback (most recent call last):
...
TypeError: Incompatible alphabets IUPACProtein() and IUPACUnambiguousDNA()
```

➔ To do this, first give both sequences generic alphabets

```
>>> from Bio.Alphabet import generic_alphabet
>>> protein_seq.alphabet = generic_alphabet
>>> dna_seq.alphabet = generic_alphabet
>>> protein_seq + dna_seq

Seq('EVRNAKACGT', Alphabet())
```

➔ Adding a generic nucleotide seq. to an unambiguous IUPAC DNA seq.

```
>>> from Bio.Alphabet import generic_nucleotide
>>> from Bio.Alphabet import IUPAC
>>> nuc_seq = Seq("GATCGATGC", generic_nucleotide)
>>> dna_seq = Seq("ACGT", IUPAC.unambiguous_dna)
>>> nuc_seq + dna_seq

Seq(GATCGATGCACGT', NucleotideAlphabet())
```

Concatenating or adding sequences (2)

➔ Many sequences to add together:

```
>>> from Bio.Alphabet import generic_dna
>>> list_of_seqs = [Seq("ACGT", generic_dna), Seq("AACC", generic_dna), Seq("GGTT", generic_dna)]
>>> concatenated = Seq("", generic_dna)
>>> for s in list_of_seqs:
... concatenated += s
...
>>> concatenated
Seq(ACGTAACCGGTT', DNAAlphabet())
```

➔ more elegant approach using sum function with its optional start value argument

```
>>> from Bio.Alphabet import generic_dna
>>> list_of_seqs = [Seq("ACGT", generic_dna), Seq("AACC", generic_dna), Seq("GGTT", generic_dna)]
>>> sum(list_of_seqs, Seq("", generic_dna))
Seq(ACGTAACCGGTT', DNAAlphabet())
```

Changing case

➔ very useful upper and lower methods for changing the case

```
>>> from Bio.Alphabet import generic_dna
>>> dna_seq = Seq("acgtACGT", generic_dna)
>>> dna_seq
Seq('acgtACGT', DNAAlphabet())
```

```
>>> dna_seq.upper()
Seq(ACGTACGT', DNAAlphabet())
>>> dna_seq.lower()
Seq(acgtacgt, DNAAlphabet())
```

```
>>> "GTAC" in dna_seq
False
>>> "GTAC" in dna_seq.upper()
True
```

```
>>> from Bio.Alphabet import IUPAC
>>> dna_seq = Seq("ACGT", IUPAC.unambiguous_dna)
>>> dna_seq
Seq('ACGT', IUPACUnambiguousDNA())
>>> dna_seq.lower()
Seq('acgt', DNAAlphabet())
```

Nucleotide sequences and (reverse) complements

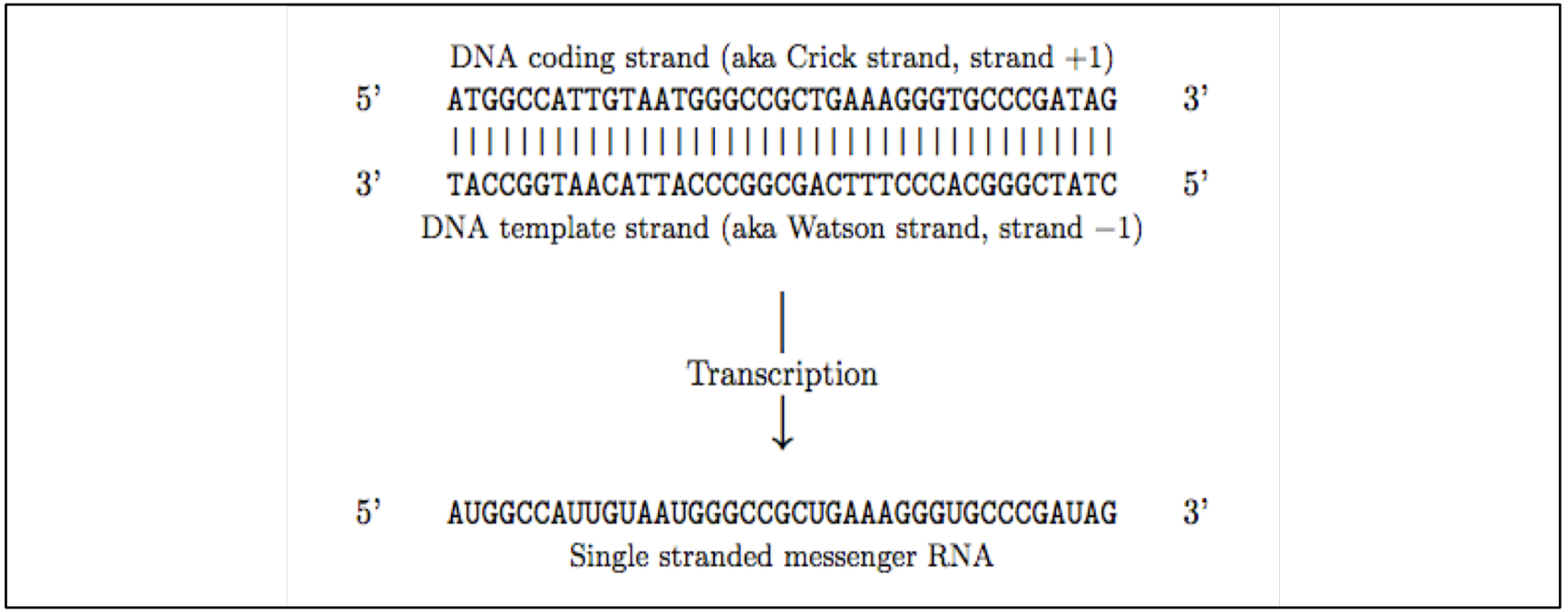
➔ For nucleotide sequences, you can easily obtain the complement or reverse complement of a Seq object using its built-in methods:

```
>>> from Bio.Alphabet import IUPAC
>>> my_seq = Seq("GATCGATGGGCCTATATAGGATCGAAAATCGC", IUPAC.unambiguous_dna)
>>> my_seq
Seq('GATCGATGGGCCTATATAGGATCGAAAATCGC', IUPACUnambiguousDNA())
```

```
>>> my_seq.complement()
Seq('CTAGCTACCCGGATATATCCTAGCTTTTAGCG', IUPACUnambiguousDNA())
>>> my_seq.reverse_complement()
Seq('GCGATTTTCGATCCTATATAGGCCCATCGATC', IUPACUnambiguousDNA())
>>> my_seq[::-1]
Seq('CGCTAAAAGCTAGGATATATCCGGGTAGCTAG', IUPACUnambiguousDNA())
```

Transcription

- Consider the following:



- The actual biological transcription process works from the template strand, doing a reverse complement (TCAG -> CUGA) to give the mRNA. However, in Biopython and bioinformatics in general, we typically work directly with the coding strand because this means we can get the mRNA sequence just by switching T -> U

Transcription (2)

- Match the figure above
  - remember by convention nucleotide sequences are normally read from the 5’ to 3’ direction, while in the figure the template strand is shown reversed.

```
>>> from Bio.Alphabet import IUPAC
>>> coding_dna = Seq("ATGGCCATTGTAATGGGCCGCTGAAAGGGTGCCCGATAG", IUPAC.unambiguous_dna)
>>> coding_dna
Seq('ATGGCCATTGTAATGGGCCGCTGAAAGGGTGCCCGATAG', IUPACUnambiguousDNA())
>>> template_dna = coding_dna.reverse_complement()
>>> template_dna
Seq(‘CTATCGGGCACCCTTTCAGCGGCCCATTACAATGGCCAT’, IUPACUnambiguousDNA())
```

- Transcribe the coding strand into corresponding mRNA, using Seq object's built in transcribe method (switch T->U and adjust the alphabet)

```
>>> coding_dna
Seq(‘ATGGCCATTGTAATGGGCCGCTGAAAGGGTGCCCGATAG’, IUPACUnambiguousDNA())
>>> messenger_rna = coding_dna.transcribe()
>>> messenger_rna
Seq('AUGGCCAUUGUAAUGGGCCGCUGAAAGGGUGCCCGAUAG', IUPACUnambiguousRNA())
```

Transcription (3) (added in Biopython 1.49)

➔ Do a true biological transcription starting with the template strand:

```
>>> template_dna.reverse_complement().transcribe()
Seq('AUGGCCAUUGUAAUGGGCCGCUGAAAGGGUGCCCGAUAG', IUPACUnambiguousDNA())
```

➔ The Seq object also includes a back-transcription method for going from the mRNA to the coding strand of the DNA:

```
>>> from Bio.Alphabet import IUPAC
>>> messenger_rna = Seq("AUGGCCAUUGUAAUGGGCCGCUGAAAGGGUGCCCGAUAG", IUPAC.unambiguous_rna)
>>> messenger_rna
Seq('AUGGCCAUUGUAAUGGGCCGCUGAAAGGGUGCCCGAUAG', IUPACUnambiguousRNA())
>>> messenger_rna.back_transcribe()
Seq('ATGGCCATTGTAATGGGCCGCTGAAAGGGTGCCCGATAG', IUPACUnambiguousDNA())
```

Translation

➔ Translate mRNA into the corresponding protein sequence

```
>>> from Bio.Alphabet import IUPAC
>>> messenger_rna = Seq("AUGGCCAUUGUAAUGGGCCGCUGAAAGGGUGCCCGAUAG", IUPAC.unambiguous_rna)
>>> messenger_rna
Seq('AUGGCCAUUGUAAUGGGCCGCUGAAAGGGUGCCCGAUAG', IUPACUnambiguousRNA())
>>> messenger_rna.translate()
Seq('MAIVMGR*KGAR*', HasStopCodon(IUPACProtein(), '*'))
```

➔ You can also translate directly from the coding strand DNA sequence:

```
>>> from Bio.Alphabet import IUPAC
>>> coding_dna = Seq("ATGGCCATTGTAATGGGCCGCTGAAAGGGTGCCCGATAG", IUPAC.unambiguous_dna)
>>> coding_dna
Seq('ATGGCCATTGTAATGGGCCGCTGAAAGGGTGCCCGATAG', IUPACUnambiguousDNA())
>>> coding_dna.translate()
Seq('MAIVMGR*KGAR*', HasStopCodon(IUPACProtein(), '*'))
```

➔ Available in Biopython from the NCBI

```
>>> coding_dna.translate(table="Vertebrate Mitochondrial")
Seq('MAIVMGRWKGAR*', HasStopCodon(IUPACProtein(), '*'))
```

➔ Using the NCBI table number

```
>>> coding_dna.translate(table=2)
Seq('MAIVMGRWKGAR*', HasStopCodon(IUPACProtein(), '*'))
```

Translation (2)

- Translate nucleotides up to the first in frame stop codon, and then stop

```
>>> coding_dna.translate()
Seq('MAIVMGR*KGAR*', HasStopCodon(IUPACProtein(), '*'))
```

```
>>> coding_dna.translate(to_stop=True)
Seq('MAIVMGR', IUPACProtein())
```

```
>>> coding_dna.translate(table=2)
Seq('MAIVMGRWKGAR*', HasStopCodon(IUPACProtein(), '*'))
```

```
>>> coding_dna.translate(table=2, to_stop=True) ## the stop codon itself is not translated
Seq('MAIVMGRWKGAR', IUPACProtein())
```

- complete coding sequence CDS, (e.g. mRNA { after any splicing)
- commences with a start codon, ends with a stop codon, and has no internal in-frame stop codons
- what if your sequence uses a non-standard start codon?
- This happens a lot in bacteria, for example, the gene yaaX in E. coli K12

Translation (3)

```
>>> from Bio.Alphabet import generic_dna
>>> gene = Seq("GTGAAAAAGATGCAATCTATCGTACTCGCACTTTCCCTGGTTCTGGTCGCTCCCATGGCA" + \
... "GCACAGGCTGCGGAAATTACGTTAGTCCCGTCAGTAAAATTACAGATAGGCGATCGTGAT" + \
... "AATCGTGGCTATTACTGGGATGGAGGTCACTGGCGCGACCACGGCTGGTGGAAACAACAT" + \
... "TATGAATGGCGAGGCAATCGCTGGCACCTACACGGACCGCCGCCACCGCCGCGCCACCAT" + \
... "AAGAAAGCTCCTCATGATCATCACGGCGGTCATGGTCCAGGCAAACATCACCGCTAA",
... generic_dna)
>>> gene.translate(table="Bacterial")
Seq('VKKMQSIVLALSLVLVAPMAAQAAEITLVPSVKLQIGDRDNRGYYWDGGHWRDH...HR*',
HasStopCodon(ExtendedIUPACProtein(), '*')
>>> gene.translate(table="Bacterial", to_stop=True)
Seq('VKKMQSIVLALSLVLVAPMAAQAAEITLVPSVKLQIGDRDNRGYYWDGGHWRDH...HHR',
ExtendedIUPACProtein())
```

➔ In the bacterial genetic code GTG is a valid start codon, and while it does normally encode Valine, if used as a start codon it should be translated as methionine. This happens if you tell Biopython your sequence is a complete CDS:

```
>>> gene.translate(table="Bacterial", cds=True)
Seq('MKKMQSIVLALSLVLVAPMAAQAAEITLVPSVKLQIGDRDNRGYYWDGGHWRDH...HHR',
ExtendedIUPACProtein())
```

Translation Tables

```
>>> from Bio.Data import CodonTable
>>> standard_table = CodonTable.unambiguous_dna_by_name["Standard"]
>>> mito_table = CodonTable.unambiguous_dna_by_name["Vertebrate Mitochondrial"]
```

```
>>> from Bio.Data import CodonTable
>>> standard_table = CodonTable.unambiguous_dna_by_id[1]
>>> mito_table = CodonTable.unambiguous_dna_by_id[2]
```

```
>>> print(standard_table)
Table 1 Standard, SGC0
```

	T	C	A	G	
T	TTT F	TCT S	TAT Y	TGT C	T
T	TTC F	TCC S	TAC Y	TGC C	C
T	TTA L	TCA S	TAA Stop	TGA Stop	A
T	TTG L(s)	TCG S	TAG Stop	TGG W	G
C	CTT L	CCT P	CAT H	CGT R	T
C	CTC L	CCC P	CAC H	CGC R	C
C	CTA L	CCA P	CAA Q	CGA R	A
C	CTG L(s)	CCG P	CAG Q	CGG R	G
A	ATT I	ACT T	AAT N	AGT S	T
A	ATC I	ACC T	AAC N	AGC S	C
A	ATA I	ACA T	AAA K	AGA R	A
A	ATG M(s)	ACG T	AAG K	AGG R	G
G	GTT V	GCT A	GAT D	GGT G	T
G	GTC V	GCC A	GAC D	GGC G	C
G	GTA V	GCA A	GAA E	GGA G	A
G	GTG V	GCG A	GAG E	GGG G	G

Translation Tables (2)

```
>>> print(mito_table)
Table 2 Vertebrate Mitochondrial, SGC1
```

	T	C	A	G	
T	TTT F	TCT S	TAT Y	TGT C	T
T	TTC F	TCC S	TAC Y	TGC C	C
T	TTA L	TCA S	TAA Stop	TGA W	A
T	TTG L	TCG S	TAG Stop	TGG W	G
C	CTT L	CCT P	CAT H	CGT R	T
C	CTC L	CCC P	CAC H	CGC R	C
C	CTA L	CCA P	CAA Q	CGA R	A
C	CTG L	CCG P	CAG Q	CGG R	G
A	ATT I(s)	ACT T	AAT N	AGT S	T
A	ATC I(s)	ACC T	AAC N	AGC S	C
A	ATA M(s)	ACA T	AAA K	AGA Stop	A
A	ATG M(s)	ACG T	AAG K	AGG Stop	G
G	GTT V	GCT A	GAT D	GGT G	T
G	GTC V	GCC A	GAC D	GGC G	C
G	GTA V	GCA A	GAA E	GGA G	A
G	GTG V(s)	GCG A	GAG E	GGG G	G

```
>>> mito_table.stop_codons
['TAA', 'TAG', 'AGA', 'AGG']
```

```
>>> mito_table.start_codons
['ATT', 'ATC', 'ATA', 'ATG', 'GTG']
```

```
>>> mito_table.forward_table["ACG"]
'T'
```

Comparing Seq objects

- Meaning of the letters in a sequence are context dependent
- The letter “A" could be part of a DNA, RNA or protein sequence.
- Comparing two Seq objects could mean considering both the sequence strings and the alphabets
- Compare the sequences as string:

```
>>> from Bio.Seq import Seq
>>> from Bio.Alphabet import IUPAC
>>> seq1 = Seq("ACGT", IUPAC.unambiguous_dna)
>>> seq2 = Seq("ACGT", IUPAC.ambiguous_dna)
>>> str(seq1) == str(seq2)
True

>>> str(seq1) == str(seq1)
True
```

- Sequence comparison only looks at the sequence, ignoring alphabet

```
>>> seq1 == seq2
True
>>> seq1 == "ACGT"
True
```

- Note if you compare sequences with incompatible alphabets (e.g. DNA vs RNA, or nucleotide versus protein), then you will get a warning but for the comparison itself only the string of letters in the sequence is used:

```
>>> from Bio.Seq import Seq
>>> from Bio.Alphabet import generic_dna, generic_protein
>>> dna_seq = Seq("ACGT", generic_dna)
>>> prot_seq = Seq(``ACGT", generic_protein)
>>> dna_seq == prot_seq
BiopythonWarning: Incompatible alphabets DNAAlphabet() and ProteinAlphabet()
True
```

- WARNING: Older versions of Biopython instead used to check if the Seq objects were the same object in memory.
- Important if you need to support scripts on both old and new versions of Biopython.
- Make the comparison explicit by wrapping your sequence objects with either str(...) for string based comparison or id(...) for object instance based comparison.

MutableSeq objects

➔ The Seq object is “read only", or in Python terminology, immutable

```
>>> from Bio.Seq import Seq
>>> from Bio.Alphabet import IUPAC
>>> my_seq = Seq("GCCATTGTAATGGGCCGCTGAAAGGGTGCCCGA", IUPAC.unambiguous_dna)
```

➔ Observe what happens if you try to edit the sequence:

```
>>> my_seq[5] = "G »
Traceback (most recent call last):
TypeError: 'Seq' object does not support item assignment
```

➔ However, you can convert it into a mutable sequence (a MutableSeq object) and do pretty much anything you want with it:

```
>>> mutable_seq = my_seq.tomutable()
>>> mutable_seq
MutableSeq('GCCATTGTAATGGGCCGCTGAAAGGGTGCCCGA', IUPACUnambiguousDNA())
```

➔ Alternatively, you can create a MutableSeq object directly from a string:

```
>>> from Bio.Seq import MutableSeq
>>> from Bio.Alphabet import IUPAC
>>> mutable_seq = MutableSeq("GCCATTGTAATGGGCCGCTGAAAGGGTGCCCGA", IUPAC.unambiguous_dna)
>>> mutable_seq
MutableSeq('GCCATTGTAATGGGCCGCTGAAAGGGTGCCCGA', IUPACUnambiguousDNA())
```

➔ Either way will give you a sequence object which can be changed:

```
>>> from Bio.Seq import MutableSeq
>>> from Bio.Alphabet import IUPAC
>>> mutable_seq = MutableSeq("GCCATTGTAATGGGCCGCTGAAAGGGTGCCCGA", IUPAC.unambiguous_dna)
>>> mutable_seq
MutableSeq('GCCATTGTAATGGGCCGCTGAAAGGGTGCCCGA', IUPACUnambiguousDNA())
>>> mutable_seq[5] = "C"
>>> mutable_seq
MutableSeq('GCCATCGTAATGGGCCGCTGAAAGGGTGCCCGA', IUPACUnambiguousDNA())
>>> mutable_seq.remove("T")
>>> mutable_seq
MutableSeq('GCCACGTAATGGGCCGCTGAAAGGGTGCCCGA', IUPACUnambiguousDNA())
>>> mutable_seq.reverse()
>>> mutable_seq
MutableSeq('AGCCCGTGGGAAAGTCGCCGGGTAATGCACCG', IUPACUnambiguousDNA())
```

➔ Note that unlike the Seq object, the MutableSeq object's methods like reverse_complement() and reverse() act in-situ!

```
>>> new_seq = mutable_seq.toseq()
>>> new_seq
Seq('AGCCCGTGGGAAAGTCGCCGGGTAATGCACCG', IUPACUnambiguousDNA())
```

UnknowSeq objects

- Subclass of the basic Seq object
- Represent a sequence where we know the length, but not the actual letters making it up.

```
>>> from Bio.Seq import UnknownSeq
>>> unk = UnknownSeq(20)
>>> unk
UnknownSeq(20, alphabet = Alphabet(), character = '?')
>>> print(unk)
????????????????????
>>> len(unk)
20
```

- Specify an alphabet, meaning for nucleotide sequences the letter defaults to “N" and for proteins “X", rather than just “?"

```
>>> from Bio.Seq import UnknownSeq
>>> from Bio.Alphabet import IUPAC
>>> unk_dna = UnknownSeq(20, alphabet=IUPAC.ambiguous_dna)
>>> unk_dna
UnknownSeq(20, alphabet = IUPACAmbiguousDNA(), character = 'N')
>>> print(unk_dna)
NNNNNNNNNNNNNNNNNNNN
```